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- 5 Effects of combination between web density and size of spider mite on predation by a generalist and a
- 6 specialist phytoseiid mite
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Abstract

Complicated three-dimensional webs of silk threads produced by *Tetranychus* spider mites provide protection from predation by many generalist phytoseiid mite species. Accessibility to prey may be the most significant criterion for successful predation in complicated webs. To assess the protective effects of combination between web size and density, we compared predation on eggs of the two-spotted spider mite, *Tetranychus urticae*, by a generalist (*Euseius sojaensis*) and a specialist (*Neoseiulus womersleyi*) phytoseiid mite in the laboratory under manipulated web size and density. Web sizes negatively affected to the predation. Significant interactions were found between phytoseiid mite species and prey distribution; egg consumption by *E. sojaensis* was more in uniform distribution, but that by *N. womersleyi*, in contrast, was larger in contagious distribution. However, the egg consumption by *E. sojaensis* and *N. womersleyi* were both mitigated in larger webs. This area size depending mitigation was more effective to the predation by *E. sojaensis*. Although the mechanism of web size depending mitigation is unknown, web sizes might affect prey searching efficiency of this phytoseiid mite. Consequently, combination between web density and size are likely to affect not only a generalist *E. sojaensis* but also a specialist *N. womersleyi*.

Keywords: Web size, Web density, *Euseius sojaensis*, *Neoseiulus womersleyi*, Tetranychidae, Phytoseiidae

Introduction

Tetranychus spider mites (Acari: Tetranychidae) produce complicated three-dimensional silk webs, on which they deposit eggs, fecal pellets, and cast skins (Saito 1983). These webs provide refuge from predation by generalist phytoseiid mites by impeding and entangling them (McMurtry and Scriven 1964, McMurtry and Johnson 1965, Osakabe 1988). *Tetranychus* spider mites usually spin silk threads as they walk (Saito 1983). Therefore, larger groups of individuals construct denser, more complex webs that may confer greater protection from generalist phytoseiid mites. However, webs also provide a prominent prey-searching cue for specialist phytoseiid mites (Hislop and Prokopy 1981, Hoy and Smilanick 1981, Sabelis et al. 1984, Furuichi et al. 2005, Yano and Osakabe 2009, Shinmen et al. 2010).

Accessibility to prey (i.e., capacity to penetrate complicated webs) may be the most significant criterion for successful predation by phytoseiid mites in complicated webs produced by *Tetranychus* spider mites (Sabelis and Bakker 1992, Shimoda et al. 2009). *Euseius sojaensis* Ehara (Acari: Phytoseiidae) is a generalist phytoseiid mite (Type IV; McMurtry and Croft 1997) incapable of penetrating complicated webs (Osakabe et al. 1986, 1987; Osakabe 1988). However, Ozawa and Yano (2009) pointed out that although prey is inaccessible after the web is completely constructed, *E. sojaensis* can prey on *Tetranychus* mites at the beginning of colonization. Moreover, Yano (2012) showed that increasing the interval between the start of *Tetranychus* spider mite colonization and prey searching by *E. sojaensis* significantly mitigated predation. Accumulation of webs during this interval may provide better protection, whereas the protective function of sparse webs may be insufficient even against a generalist phytoseiid mite. Although examinations of this relationship usually assume that spider mites and their eggs are distributed throughout their webs, predation risk to eggs may be higher if those are distributed near the web's edge. In this context, predation risk would be higher when the webbing area is smaller. However, the protective effects of combination between web density and size have not been addressed virtually.

To assess the protective effects of combination between web size and density, we compared predation on eggs of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), by *E. sojaensis* and *Neoseiulus womersleyi* (Schicha) (Acari: Phytoseiidae) in the laboratory under manipulated webbing area size and aggregation of adult females involving prey egg distribution and also web density in the area.

Neoseiulus womersleyi is a specialist phytoseiid mite native to Japan that prefers *Tetranychus* mites (Type II; McMurtry and Croft 1997).

Materials and methods

Mites

Neoseiulus womersleyi were collected from roses (Rosaceae) in Nara, Japan and reared on *T. urticae* on kidney bean, *Phaseolus vulgaris* L. (Leguminosae), leaves placed on water-soaked cotton in Petri dishes (9 cm in diameter). *Euseius sojaensis* were collected from kudzu vines *Pueraria lobata* (Willd) Ohwi (Leguminosae) in Kyoto and reared on tea pollen on plastic film (Parafilm, Pechiney Plastic Packaging Co., IL; 5 cm in diameter) placed on water-soaked cotton in Petri dishes. A laboratory population of *T. urticae* (the yellow-green type) from several different localities in Japan and cultured on potted kidney bean plants has been established in our laboratory for more than 7 years. This was also reared on kidney bean leaves on water-soaked cotton in Petri dishes for several generations before use in the experiments.

Petri dishes were placed in transparent plastic containers and kept at $25 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ relative humidity (RH), with a photoperiod of 16L8D. All experiments were conducted under the same conditions as rearing. We used 2 to 3-day-old mated females of *T. urticae*, *N. womersleyi*, and *E. sojaensis* in all experiments.

Experimental design

We prepared 10×10 -mm or 15×15 -mm leaf squares cut from kidney bean leaves, excluding the main vein, as experimental patches. We pressed four (10 mm) or two (15 mm) leaf squares onto water soaked cotton in each Petri dish (9 cm in diameter). We put two wet cotton threads on each leaf square to divide it into four experimental quadrats. Four *T. urticae* females were then introduced individually into each quadrat (uniform distribution; Fig. 1a) or together into one quadrat (contagious distribution; Fig. 1b) in a leaf square.

After 24 h, we recorded the number of eggs laid in each quadrat; then, we carefully removed females

and wet cotton threads so as not to destroy the webs. An adult female of *E. sojaensis* or *N. womersleyi* was introduced at the center of each leaf square. To ensure they were in a uniformly starved condition, the phytoseiid mites were kept individually in 1.5-ml sample tubes with a water droplet for 48 h before the experiments. After 24 h, we recorded the number of *T. urticae* eggs consumed. If a phytoseiid mite escaped from the leaf square during an experiment, the data were excluded from the following analyses.

The area covered by complicated webs was in direct proportion to the area walked by *T. urticae* females, which ranged from 50 to 100 mm² over 10–90 min in a laboratory experiment by Saito (1977). In our experiments, *T. urticae* adult females were allowed to walk freely in a 25-mm² (in the case of the 10-mm square) or 56.25-mm² (15-mm square) quadrat for 24 h. Therefore, quadrat sizes roughly corresponded to the area of complicated webs produced by *T. urticae*.

Replications (leaf squares) of uniform and contagious distributions against *E. sojaensis* numbered 15 and 12, respectively, for 10-mm squares and 11 and 12, respectively, for 15-mm squares. Replications of uniform and contagious distributions against *N. womersleyi* numbered 16 and 8, respectively, for 10-mm squares, and 20 and 16, respectively, for 15-mm squares.

Data analysis

We compared the numbers of eggs deposited in leaf squares among square sizes and distributions of introduced *T. urticae* females using a Wald test in a generalized linear model (GLM; Poisson error). Effects of area sizes and distribution of prey eggs on egg consumption by phytoseiid mites were analyzed using a linear model. Prior to this analysis, proportion of eggs consumed by phytoseiid mites were transformed to empirical logit. All analyses were conducted in R v. 2.15.2 (R Core Development Team 2012).

Results

The number of eggs produced by four *T. urticae* females ranged from 42 to 56 (Table 1), and more on 15-mm than on 10-mm squares (Table 2 A). The distribution pattern of *T. urticae* females also significantly affected egg production: more in uniform than contagious distribution (Table 2 D).

No significant difference was detected in prey egg consumption between phytoseiid mite species (Table 3 P). Whereas, the area sizes negatively affected to the predation; egg predation rate was larger on 10-mm leaf squares than 15-mm leaf squares (Fig. 2, Table 3 A), and this trend was clear in *E. sojaensis* in comparison with *N. womersleyi* (Table 3 P×A). Effects of prey distribution on egg consumption were marginal (Table 3 D). However, egg consumption by *E. sojaensis* decreased in contagious distribution of prey in comparison with uniform distribution on 10-mm leaf squares, but that by *N. womersleyi*, in contrast, increased in contagious distribution (Fig. 2, Table 3 P×D). No interaction was detected between leaf size and distribution of prey eggs (Table 3 A×D).

Discussion

The egg consumption rates demonstrate opposite effects of aggregation of prey eggs to predation by *E. sojaensis* and *N. womersleyi* in smaller area (10-mm squares). Contagious distribution decreased predation by *E. sojaensis*, indicating that dense webs produced by aggregated females increased protection effects. This may reflect weakness of *E. sojaensis* to complicated webs of *Tetranychus* mites (Osakabe et al. 1986, 1987; Osakabe 1988). In contrast, predation by *N. womersleyi* increased in contagious distribution. Dense webs did not affect the degree of protection from predation by *N. womersleyi*. Instead, the dense webs might be exploited as a prey-searching cue because this phytoseiid mite follows *T. urticae* trail including threads (Yano and Osakabe 2009, Shinmen et al. 2010) and their prey preference is strongly affected by spider mite webs (Fruichi et al. 2005). Moreover, the extent of *N. womersleyi*'s prey consumption may be due to its ability to cut silken threads while moving within the webs (Shimoda et al. 2010).

In contrast, predation risks by *E. sojaensis* in uniform distribution and by *N. womersleyi* in contagious distribution were both mitigated in larger area (15-mm squares). This area size depending mitigation was more effective to the predation by *E. sojaensis*. Denser webs conferred stronger protection from predation by *E. sojaensis* on 10-mm leaf squares as described above. However, the web density was lower on 15-mm than 10-mm leaf squares in the same distribution because webs were created by the same number of individuals. This demonstrated that predation by *E. sojaensis* was affected by the combination between web density and size. Interestingly, egg consumption by *N. womersleyi* in contagious distribution

151 also decreased in 15-mm leaf squares. Although the mechanism of this phenomenon is unknown, web
152 sizes might affect prey searching efficiency of this phytoseiid mite. Therefore, combination between web
153 density and size are likely to affect not only a generalist *E. sojaensis* but also a specialist *N. wormersleyi*.
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210 Figure legend

211

212 Fig. 1 Design for uniform (a) and contagious (b) distribution treatment of *Tetranychus urticae* females
 213 (solid circles) over four quadrats separated by wet cotton threads on kidney bean leaf squares
 214 (10- or 15-mm square)

215

216 Fig. 2 Predation rates on uniformly and contagiously distributed prey eggs by *Euseius sojaensis* (A, B)
 217 and *Neoseiulus womersleyi* (C, D) on 15-mm leaf squares (A, C) and 10-mm leaf squares (B, D)
 218 for 24 h. Vertical lines at the top of bars represents standard errors (SE). The mean and SE values
 219 were calculated with arc sin transformed predation rates of replications.

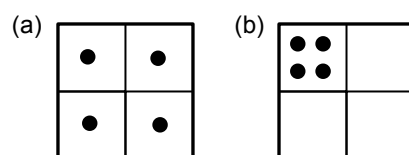


Fig. 1

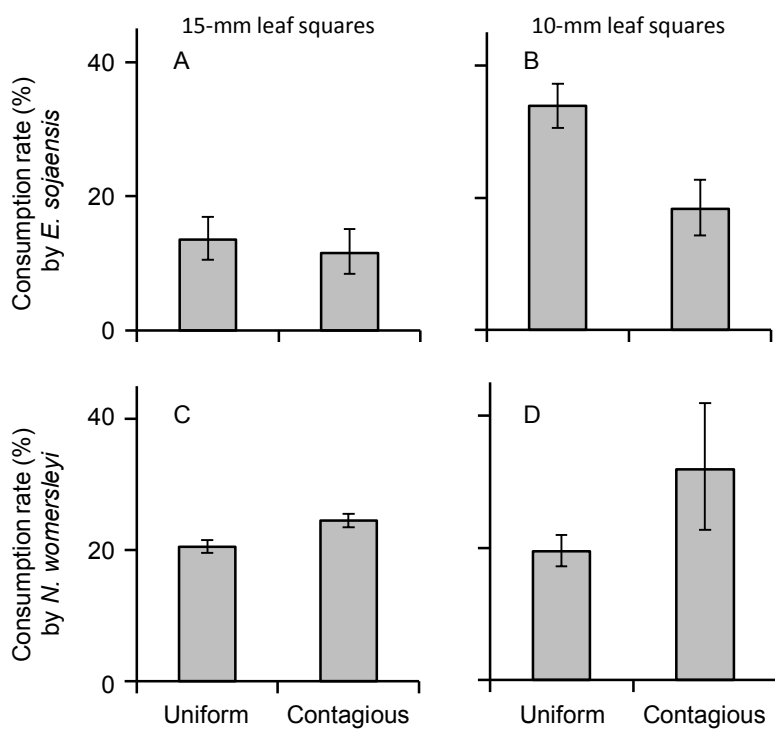


Fig. 2